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VOL. 24, SEC. C. APRIL, 1946 NUMBER 2

THE EFFECT OF DDT ON THE STEM RUST REACTION OF KHAPLI WHEAT[1]

BY T. JOHNSON[2]

Abstract

Seedling leaves of Khapli wheat—a variety highly resistant to physiologic races of wheat stem rust prevalent in North America—became susceptible to stem rust a few days after they had been sprayed with DDT (1 oz. in 5 gal. water). The response to DDT was highly specific, as only one other of the resistant wheats tested (Arnautka) showed any indication of susceptibility consequent on spraying. Specificity of response was also indicated by the development of marked chlorosis on leaves of some varieties, whereas other varieties remained unaffected.

DDT (dichlorodiphenyltrichloroethane) has recently come into use for the control of white flies and other insects in the greenhouses of the Dominion Laboratory of Plant Pathology, Winnipeg, Man. Among the plants sprayed in one of the applications were certain wheat varieties employed as differential hosts for physiologic races of stem rust of wheat (*Puccinia graminis Tritici* Erikss. & Henn.). The wheat varieties had been inoculated, in the seedling stage, with race 17 of this rust four days prior to spraying with DDT. When observations were made on rust development, 10 days after the spraying took place, it was noted that the rust pustules (uredia) on the highly resistant variety Khapli (*T. dicoccum*) were somewhat larger than the minute type 1 pustule normally produced on Khapli by race 17 and, indeed, by all other races of stem rust commonly prevalent in North America. The plants were kept for further observation and it was noted that the rust pustules on Khapli continued to increase in size until, 24 days after spraying took place, they were classified as type 4—a type of pustule associated with full susceptibility of the host.

To determine whether or not the pathogenicity of the rust had changed, spores of these large pustules were sown on the leaves of several wheat varieties including Khapli. The pustules that later developed on these varieties were typical of race 17, those on Khapli being classified as the characteristic type 1 pustule formed on that variety. Further observation showed no subsequent increase in the size of these pustules. It was therefore evident that the DDT had effected no change in the pathogenicity of the rust.

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Contribution No. 842 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] *Plant Pathologist, Dominion Laboratory of Plant Pathology, Winnipeg, Man.*

Subsequently, an experiment was made to determine the effect of DDT on infections caused by other races of wheat stem rust. Two sets of differential hosts (wheat varieties) were inoculated with races 15 and 56, respectively, on November 7. Three days later one set inoculated with each of these races was sprayed with a solution of DDT of the same concentration as that previously used for white fly control (1 oz. DDT in 1 litre of 95% ethyl alcohol added to 5 gal. water containing a small amount of Aerosol OT). The plants were sprayed as uniformly as possible to ensure that all varieties received about the same application of DDT. A control set, for each race, was sprayed with exactly the same solution except that no DDT was present.

When observations were made on rust development, nine days after the spray was applied, both races had produced considerably larger rust pustules on the Khapli plants sprayed with DDT than on those sprayed with the control solution. The pustules on the treated Khapli plants were recorded as type 3, for race 56, and 3−, for race 15, whereas those on the control plants were in both cases recorded as type 1−. Infections on treated and control plants of Khapli are shown in Fig. 1 as they appeared 13 days after spraying.

Examination of the infection types present on the other differential hosts made it clear that response to DDT is highly specific in that the presence or absence of any response depends on the host variety infected. Among the differential hosts were four varieties that possess about the same degree of resistance as Khapli to race 56, namely, Arnautka, Mindum, Spelmars, and Einkorn. Only on Arnautka was there any indication of larger pustules on the plants treated with DDT than on the control plants.

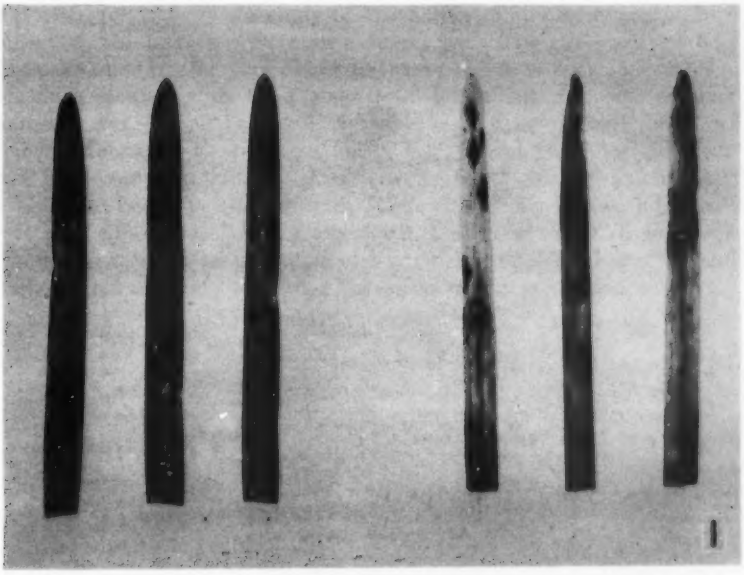
It was evident too, from examination of the treated plants, that some wheat varieties show more tolerance towards DDT than others. Leaves of the varieties Arnautka, Spelmars, and Khapli developed a pronounced chlorosis in about a week after they were sprayed, whereas the varieties Kota, Mindum, and Einkorn showed no indication of chlorosis. Faint chlorosis was noted on the varieties Marquis, Kanred, Kubanka, Acme, and Vernal. As only one concentration of DDT was used in the experiments under consideration, the possibility exists that more concentrated solutions might produce a high degree of chlorosis on all varieties.

On treated Khapli leaves there appeared to be a marked relationship between chlorosis and rust development. Chlorosis progressed gradually until, in some leaves, green colour was no longer visible. The virtual disappearance of green pigment, however, did not inhibit the increase in size of the rust pustules, which grew gradually larger until the leaves withered.

EXPLANATION OF FIGURE

FIG. 1. *The effect of DDT on stem rust reaction of seedling leaves of Khapli wheat, 16 days after inoculation and 13 days after spraying with DDT.*
Left: Leaves sprayed with control solution.
Right: Leaves sprayed with DDT.

PLATE I



The immediate significance of the foregoing observations lies in the fact that the possible effect of DDT on host reactions should not be overlooked when this substance is being used for insect control. Of more fundamental import is the possibility that the influence of DDT on host reaction may open up a new approach to the study of the nature of rust resistance. In this connection, the following facts seem suggestive. The two varieties Khapli and Spelmars normally show about the same degree of resistance to race 56 and, when sprayed with DDT they developed about the same amount of chlorosis. Yet Khapli became susceptible when sprayed with DDT while Spelmars did not. If, as seems likely, the general physiological effect of DDT is the same on both varieties, it seems reasonable to assume that the physiological basis of rust resistance differs either quantitatively or qualitatively in the two varieties. In any event, the observations recorded above suggest that it would be profitable to study the physiological effects of DDT on wheat varieties and to correlate these effects with rust reaction.

PHYSIOLOGIC RACES OF *PUCCINIA GRAMINIS TRITICI* IN CANADA, 1919 TO 1944[1]

By Margaret Newton and T. Johnson[2]

Abstract

During the period 1919 to 1944, 65 physiologic races were identified from a total of 4543 isolates derived from uredial collections of *Puccinia graminis* Pers. var. *Tritici* Erikss. & Henn. Forty-nine races were obtained in the Prairie Provinces from a study of 3475 isolates; 40 in Eastern Canada from 1013 isolates, and 12 in British Columbia from 55 isolates. During this period, the predominant races have shown notable fluctuations in their prevalence. Races 36, 17, and 21 were the most common races until shortly after 1930 and were largely responsible for the severe rust losses suffered by Marquis and other common wheats during the decade preceding that year. Races 34 and 49 were collected frequently from 1927 to about 1935. All of these races diminished greatly in their prevalence between 1930 and 1936, whereas race 56, which was first collected in Canada in 1931, has become the predominant race since 1934. It was this race that played a major part in the stem rust epiphytotic of 1935. Another recent change in the racial population was a recrudescence in 1940 of race 17, which for several previous years had been of minor importance. In 1941 this race challenged the pre-eminent position of race 56 but receded again in succeeding years to minor significance.

The distribution of races is somewhat similar but not identical in different parts of Canada. Races 36 and 21 have been relatively more common in the Prairie Provinces than in Eastern Canada, while the contrary is true of race 38. Only about a dozen of the 65 races collected in Canada have thus far assumed much economic importance, a few others may be considered of minor significance, but at least two-thirds of the races have been found only occasionally and have, for reasons not fully understood, failed to gain even a limited distribution.

A comparison of the number of physiologic races collected in Eastern Canada and the Prairie Provinces, respectively, has indicated a somewhat greater variety of physiologic races in the former region, a condition that may perhaps be explained by the presence of the common barberry in many localities in Eastern Canada.

Introduction

Specialization in *Puccinia graminis* Pers. var. *Tritici* Erikss. & Henn. was first reported by Stakman and Piemeisel in 1917 (21, 22). The first investigations on the specialization of this rust in Canada were undertaken by the senior author in 1918 at the suggestion and with the advice of the late Professor W. P. Fraser, and were continued by her until April, 1922 (9, 10). From 1922 to 1924 studies on physiologic races of wheat stem rust in the Prairie Provinces were continued by Professor Fraser (1, p. 45; 2, pp. 40–41; 3, p. 68; 15, p. 100). From 1925 until the present time, surveys of the prevalence of physiologic races have been carried out annually by the present writers. The work done during the period 1919 to 1930 was fully reported in 1932 (12) and later a brief account was given of the races isolated up to 1936 (11). As work carried out subsequent to 1936 has never been placed on

[1] *Manuscript received November 5, 1945.*

Contribution No. 837 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] *Senior Plant Pathologist and Plant Pathologist, respectively, Dominion Laboratory of Plant Pathology, Winnipeg, Man.*

record and as, moreover, records are now available on the distribution of physiologic races in the Prairie Provinces of Canada for a period of more than a quarter of a century, it seemed desirable to bring together in one publication the information now on hand.

During the seven years, from 1919 to 1925, the study of the distribution of physiologic races was almost entirely confined to the three Prairie Provinces of Western Canada. From that time onward attempts were made to extend the survey of physiologic races to other parts of Canada with the result that, in most years, collections from all the provinces have been studied.

Procedure and Methods

In securing rust collections the writers are indebted to numerous individuals and institutions throughout Canada. In the three Prairie Provinces, surveys have been conducted annually by the staffs of the laboratories of plant pathology at Winnipeg, Man., Saskatoon, Sask., and Edmonton, Alta. Rust collections from other parts of the country have been made chiefly by individuals connected with the various laboratories of plant pathology and agricultural colleges. Collections from the chief cereal growing areas of Canada were facilitated by the maintenance of uniform rust nursery plots of cereal varieties at experimental farms and stations in these areas during the periods 1920 to 1929 and 1936 to 1944. During the earlier period, while the nurseries were relatively few, annual visits were made to them. During the latter period, when the nurseries became numerous and widely distributed and visits could no longer be made to them, small sheaves of each of the varieties grown were shipped to the Winnipeg laboratory for examination.

Methods of inoculation with urediospore collections and the procedure followed in the identification of physiologic races have been described in detail elsewhere (12, 18).

During most of the period covered by the surveys, isolation of physiologic races from aeciospores has been very limited. Very few of the earlier attempts to establish cultures from aeciospores were successful, chiefly because the aecia had to be shipped long distances and methods of inoculation suitable to aeciospores in a more or less dried-out condition had not been worked out. In more recent years, however, a simple and relatively satisfactory method of inoculation has been devised. Briefly, this consists in cutting out individual aecial pustules or leaf areas bearing numerous aecia and pressing these firmly against a wet blotting paper in a Petri dish cover, with the aecia facing outward, until the leaf sections adhere to the paper. The aecia are then sprayed with a fine spray from an atomizer and suspended above a glass slide in a Petri dish bottom over night. On the following morning it is usually found that large numbers of aeciospores have been discharged on the glass slide. The aeciospores so discharged are applied to seedling cereal leaves by drawing the slide gently over the leaf surfaces. If any appreciable germination of the spores takes place, a reasonably good infection is ensured.

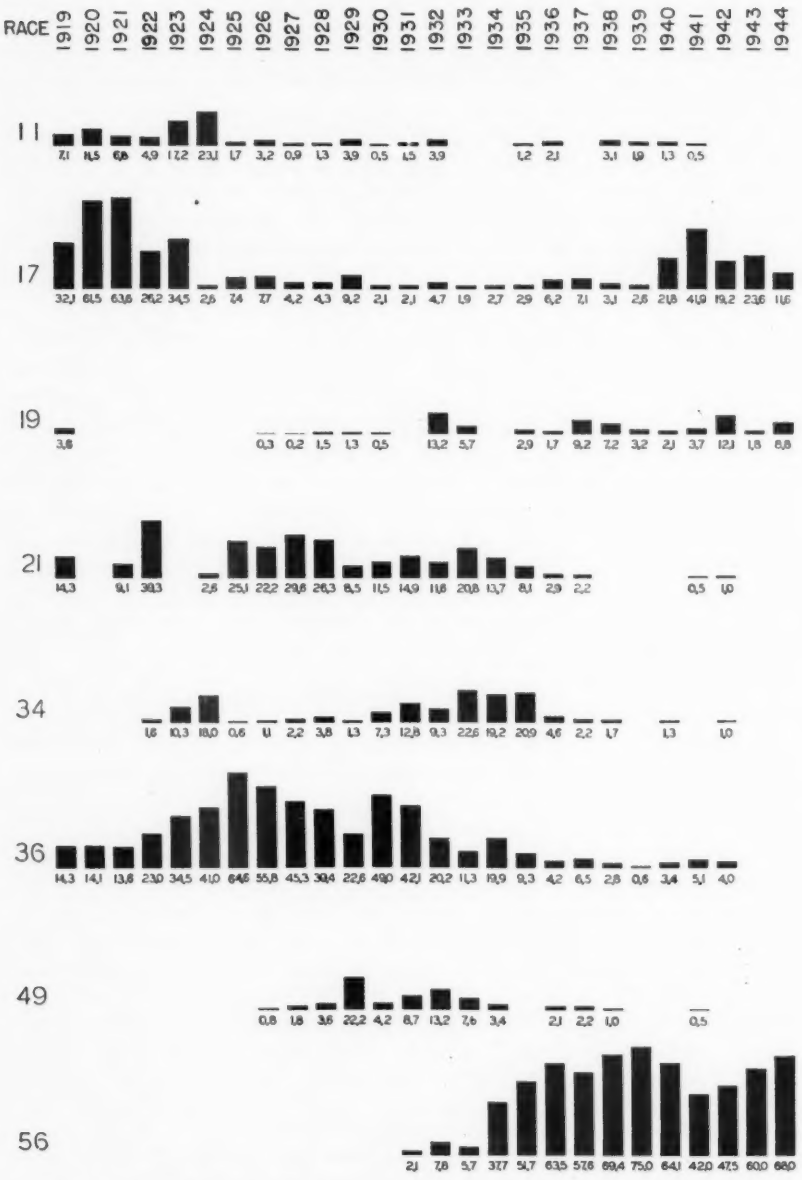


FIG. 1. *Annual frequency of occurrence of the more important physiologic races of wheat stem rust isolated in Canada during the period 1919 to 1944, expressed in per cent of the total isolates for each year. In the above chart, certain similar races recorded separately in Table I, are recorded as one race, i.e., race 32 is included with race 11, race 29 with race 17, races 3 and 18 with race 36, and race 125 with race 56.*

Results of the Surveys

The annual prevalence of physiologic races is shown in Table I and that of the more important physiologic races is represented diagrammatically in Fig. 1. It is clear that, when surveys were first undertaken in 1919, and for several years thereafter, there were two predominant groups of physiologic races. One of these groups is composed of races 3, 18, and 36, which, in the earlier years, were recorded as distinct races, but were subsequently classified as a single race (race 36) owing to their close similarity to each other. The other group consists of races 17 and 29, which, for the same reason, were grouped together as race 17. Another race of considerable importance in those early years was race 21. All of these races were characterized by the ability to attack heavily the hard red spring wheats then grown in Canada and the north central United States, namely, varieties such as Marquis, Red Fife, Preston, Ruby, Haynes Bluestem, and others. It was undoubtedly these races that were chiefly responsible for the recurrent damage to hard red spring wheat—damage so severe that farmers in certain areas of Manitoba and North Dakota were forced to abandon these wheats in favour of the more rust resistant durum wheat.

Races 36, 17, and 21 maintained their predominance until the early thirties but as early as the late twenties other races were assuming importance. Races 34 and 49 began to be common about 1927 and remained so until about 1935. Race 38 has been one of the common races, especially in Eastern Canada, since 1926.

Between 1930 and 1936 notable changes took place in the prevalence of the common races. During this period, races 17, 21, 34, 36, and 49 were found less and less frequently, whereas race 56, which was first collected in the field in Canada in 1931, had become three years later the predominant race, a position it has maintained up to the present time. Since the rise of race 56 to pre-eminence, the only pronounced change in racial population was the sudden increase, in 1940, in the prevalence of race 17 which, in 1941, challenged the predominant position of race 56. Subsequent surveys, however, have shown a sharp decrease in the prevalence of race 17.

The above outline, though based largely on the results of surveys in the Prairie Provinces, is also, in general, applicable to other parts of the country. Races that are important over a period of years in the Prairie region are also common elsewhere in Canada during the same period. No absolute agreement, however, exists between the predominance of certain races in the Prairie Provinces and Eastern Canada. For example, during the period 1926 to 1944, race 36, which was the most commonly collected race in the Prairie region, was in third place in the Maritime Provinces and in Ontario and Quebec, and race 21, which was in third place in the Prairie region, took only fifth place in the eastern regions. Still more striking is the relative prevalence of race 38 in the east and the west. This race, which was in second place in both of

TABLE I

DISTRIBUTION BY GEOGRAPHICAL AREAS OF PHYSIOLOGIC RACES OF *Puccinia graminis* VAR. *Tritici* COLLECTED ON CEREALS AND GRASSES FROM 1919 TO 1944 (FIGURES REPRESENT THE NUMBER OF TIMES EACH RACE WAS COLLECTED IN EACH GEOGRAPHICAL AREA)

Race	1919	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938	1939	1940	1941	1942	1943	1944	Total
											Maritime Provinces																
1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	1
11	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	2
14	–	–	–	–	–	–	–	–	–	1	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2
15	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2
17	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	1	–	1	–	–	–	–	–	1	2	7
19	–	–	–	–	–	–	–	1	1	1	2	–	–	–	–	–	–	–	3	3	–	–	1	–	–	1	13
21	–	–	–	–	–	–	–	13	3	6	3	1	–	–	–	–	3	–	–	–	–	–	–	–	–	–	29
23	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	1
29	–	–	–	–	–	–	–	2	–	–	2	–	–	–	–	–	–	3	4	7	–	9	6	1	2	–	36
30	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
32	–	–	–	–	–	–	–	2	–	–	2	–	–	–	–	–	–	–	–	4	–	–	–	–	–	–	8
34	–	–	–	–	–	–	–	–	–	2	–	–	–	–	1	1	7	1	3	2	–	–	–	–	–	–	17
35	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
36	–	–	–	–	–	–	–	30	6	5	3	4	1	–	–	–	4	–	2	1	–	–	–	–	–	–	56
38	–	–	–	–	–	–	–	6	9	13	10	4	1	–	5	–	5	7	7	11	3	7	–	3	2	–	93
39	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	1	1	–	–	–	–	1	–	6
49	–	–	–	–	–	–	–	1	–	–	2	1	–	–	–	–	–	–	2	2	–	–	–	–	–	–	8
50	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	1	–	–	–	–	–	–	–	2
56	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1	10	14	24	61	21	16	4	1	4	5	162
64	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
74	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
75	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
77	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
96	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	1
98	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	1
125	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	1	–	–	–	–	2
183	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	1

TABLE I—*Continued*

DISTRIBUTION BY GEOGRAPHICAL AREAS OF PHYSIOLOGIC RACES OF *Puccinia graminis* VAR. *Tritici* COLLECTED ON CEREALS AND GRASSES FROM 1919 TO 1944 (FIGURES REPRESENT THE NUMBER OF TIMES EACH RACE WAS COLLECTED IN EACH GEOGRAPHICAL AREA)—*Continued*

Race	1919	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938	1939	1940	1941	1942	1943	1944	Total
											Ontario and Quebec																
1	–	–	–	–	–	–	–	–	–	–	1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2
9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1
11	–	–	–	–	–	–	–	–	–	1	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	3
14	–	–	–	–	–	–	–	1	1	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3
15	–	–	–	–	–	–	–	–	–	3	1	–	–	–	–	–	–	–	–	–	1	1	–	–	–	–	6
17	–	–	–	–	–	–	–	–	2	–	2	–	–	–	–	–	–	1	1	–	–	–	–	1	2	2	11
19	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	3	3	1	–	2	2	2	4	18
21	–	–	–	–	–	–	2	10	11	3	4	1	3	2	2	–	2	1	1	–	–	–	–	1	–	–	43
23	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	1
29	–	–	–	–	–	–	–	7	–	2	–	–	–	–	–	–	–	4	–	1	2	9	19	3	3	4	54
30	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
32	–	–	–	–	–	–	–	4	2	–	3	–	–	–	–	–	–	1	–	–	1	–	–	–	–	–	11
33	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
34	–	–	–	–	–	–	–	1	1	2	1	–	2	–	–	–	5	1	–	–	–	3	–	1	–	–	17
36	–	–	–	–	–	–	4	18	13	9	1	6	3	3	1	–	2	3	2	–	–	–	1	–	–	–	66
38	–	–	–	–	–	–	–	11	4	10	19	8	1	2	1	–	–	13	2	6	5	1	4	7	6	1	101
39	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	1	1	–	–	–	1	–	–	6
48	–	–	–	–	–	–	–	1	–	–	1	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	3
49	–	–	–	–	–	–	–	–	1	3	1	1	1	1	1	–	–	–	1	–	–	–	–	–	–	–	10
50	–	–	–	–	–	–	–	–	1	–	1	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	3
53	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
56	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	12	35	20	27	14	19	15	5	15	17	179
59	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	1	–	3
63	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
78	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	1
83	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
84	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
89	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	1

TABLE I—*Continued*

DISTRIBUTION BY GEOGRAPHICAL AREAS OF PHYSIOLOGIC RACES OF *Puccinia graminis* VAR. *Tritici* COLLECTED ON CEREALS AND GRASSES FROM 1919 TO 1944 (FIGURES REPRESENT THE NUMBER OF TIMES EACH RACE WAS COLLECTED IN EACH GEOGRAPHICAL AREA)—*Continued*

Race	1919	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938	1939	1940	1941	1942	1943	1944	Total
Ontario and Quebec—*Concluded*																											
92	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	1
113	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	1	–	2
125	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	–	–	–	–	1	–	–	–	4
192	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	1
Prairie Provinces																											
1	2	1	–	–	–	–	–	–	–	2	10	1	2	2	–	1	–	–	–	–	–	–	–	–	–	–	21
2	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
3	–	4	3	10	10	16	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	1	–	–	–	–	45
9	4	6	2	3	–	–	–	1	4	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	21
10	–	–	–	–	–	–	–	–	–	–	–	–	3	4	–	–	–	–	–	–	–	–	–	–	–	–	7
11	2	5	2	3	5	9	–	–	–	3	2	–	1	3	–	–	1	–	–	–	–	1	–	–	–	–	37
12	–	2	–	–	–	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7
14	–	–	–	–	–	–	–	1	15	1	4	3	3	2	4	–	–	–	–	–	1	–	–	–	–	–	34
15	1	–	–	–	–	–	–	2	8	16	–	–	–	1	–	–	–	–	1	2	–	–	1	1	–	–	33
16	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2
17	9	31	27	16	10	1	–	1	13	6	9	–	3	6	–	2	–	3	4	–	–	2	–	–	3	6	152
18	4	7	3	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	16
19	1	–	–	–	–	–	–	–	–	5	1	1	–	17	3	–	5	4	11	15	4	5	5	10	–	8	95
21	4	–	4	24	–	1	42	61	118	93	19	19	23	13	9	20	9	6	3	–	–	–	1	–	–	–	469
23	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2
24	1	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2
27	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
29	–	17	1	–	–	–	13	19	4	9	13	4	1	–	1	2	4	4	3	1	2	31	65	14	15	3	226
30	–	1	–	–	–	–	1	7	5	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	16
32	–	4	1	–	–	–	3	6	2	–	5	1	–	2	–	–	1	3	–	3	2	2	1	–	–	–	36

TABLE I—*Continued*

DISTRIBUTION BY GEOGRAPHICAL AREAS OF PHYSIOLOGIC RACES OF *Puccinia graminis* VAR. *Tritici* COLLECTED ON CEREALS AND GRASSES FROM 1919 TO 1944 (FIGURES REPRESENT THE NUMBER OF TIMES EACH RACE WAS COLLECTED IN EACH GEOGRAPHICAL AREA)—*Continued*

Race	1919	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938	1939	1940	1941	1942	1943	1944	Total
											Prairie Provinces—*Concluded*																
33	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	1
34	–	–	–	1	3	7	1	3	9	11	3	14	23	12	11	27	24	9	1	3	–	–	–	–	–	–	162
36	–	–	–	2	–	–	109	163	185	139	65	84	75	23	5	29	10	4	8	6	–	6	10	3	–	–	926
38	–	–	–	–	–	–	–	1	13	9	36	27	8	5	–	3	–	3	4	4	8	4	1	–	1	10	137
39	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	1	–	2	2	2	2	–	–	–	–	5	15
40	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
48	–	–	–	–	–	–	–	1	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2
49	–	–	–	–	–	–	–	2	7	11	65	6	16	16	3	5	–	5	1	1	–	–	1	–	–	–	139
50	–	–	–	–	–	–	–	–	3	–	5	–	2	1	2	–	–	–	–	–	–	–	–	–	–	–	13
51	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	1
52	–	–	–	–	–	–	–	1	3	6	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	11
53	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	1
56	–	–	–	–	–	–	–	–	–	–	–	–	3	10	2	41	64	98	54	112	80	105	64	40	45	68	786
63	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	1
73	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	1
80	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	1
81	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	1
86	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	1
87	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	1	–	–	–	–	–	2
92	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	1	–	–	–	–	–	2
97	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
102	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	1
123	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	1
125	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	13	3	3	7	–	1	3	–	–	–	9	39
136	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	1
139	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	1
147	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1	–	–	–	–	2
177	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	1
179	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	1

TABLE I—*Concluded*

DISTRIBUTION BY GEOGRAPHICAL AREAS OF PHYSIOLOGIC RACES OF *Puccinia graminis* VAR. *Tritici* COLLECTED ON CEREALS AND GRASSES FROM 1919 TO 1944 (FIGURES REPRESENT THE NUMBER OF TIMES EACH RACE WAS COLLECTED IN EACH GEOGRAPHICAL AREA)—*Concluded*

Race	1919	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938	1939	1940	1941	1942	1943	1944	Total
												British Columbia															
3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	1
11	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	1
14	–	–	–	–	–	–	–	–	–	–	–	–	4	–	–	–	–	–	–	–	–	–	–	–	–	–	4
21	–	–	–	–	–	–	–	–	1	1	–	1	3	–	–	–	–	–	–	–	–	–	–	–	–	–	6
23	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2
32	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	1
36	–	–	–	–	–	–	–	–	–	1	–	–	3	–	–	–	–	3	–	–	1	1	–	–	–	–	9
39	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	1
56	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	2	1	6	6	1	2	1	20
61	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	1
78	–	–	–	–	–	–	–	–	–	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4
113	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	3	–	5
Total	28	78	44	61	29	39	175	378	450	391	306	192	195	129	53	146	172	241	184	291	156	234	215	99	110	147	4543

the eastern regions, took only seventh place in the Prairie Provinces. The relatively lower incidence of this race in the Prairie Provinces is perhaps due to the fact that Marquis wheat, which is resistant to it, was for many years the prevailing wheat variety in that region.

The introduction of new wheat varieties undoubtedly had some influence on the prevalence of individual races. It has been pointed out (6, 16, 19, 20) that Ceres wheat, which came to be widely grown in the north central United States and in Manitoba from 1926 onwards, is more susceptible to race 56 than to the races prevalent at the time of its introduction; and it has been maintained (19, 20) that the susceptibility of Ceres to this race was a prime factor in the increase in its prevalence.

It cannot be doubted that the more recent introduction of stem rust resistant wheat varieties has greatly diminished the amount of stem rust inoculum in the regions in which they are grown. Whether or not their introduction has effected any change in the relative abundance of the different physiologic races is a more doubtful matter. As most of the new varieties are almost equally resistant to the various races common in North America, there is little likelihood that the new varieties would have the effect of increasing one race in preference to another. It is, however, not at all impossible that some little known or perhaps unknown race may possess ability to attack them and thereby gain greater distribution in Canada in future years.

The Role of Barberry in the Origination of New Races of Wheat Stem Rust

It has been amply demonstrated in laboratory experiments that physiologic races of wheat stem rust may, through hybridization and selfing on the barberry, produce races pathogenically different from those that were crossed or selfed (5, 8, 13, 14, 17). Undoubtedly, these same processes of crossing and selfing of races occur on naturally infected barberry. As stem rust spreads readily from barberry bushes to wheat and susceptible grasses growing in the vicinity of infected bushes, there is every reason to suppose that a considerable variety of physiologic races is being passed on every year from barberry to susceptible cereals and grasses.

In the Prairie Provinces, in which the great bulk of Canada's wheat crop is grown, barberry is virtually non-existent. In Eastern Canada and British Columbia, where scattered plantings of this shrub exist, there is a possibility that barberry may play a part in the origination of new physiologic races. Wheat, however, is not extensively grown in either of these regions, and the limited infection studies thus far carried out, confined entirely to aeciospores collected in Eastern Canada, indicate that there the rust on barberry is, for the most part, rye stem rust (*Puccinia graminis* var. *Secalis*). Of 33 collections of aecia gathered in 1944 in Ontario, Quebec, and the Maritime Provinces, 30 contained the *Secalis* variety either alone or mixed with other varieties.

The results of this study are summarized below:

Secalis variety alone	19	collections
Secalis + *Agrostidis* varieties	6	"
Secalis + *Avenae* varieties	3	"
Secalis + *Avenae* + *Agrostidis* varieties	1	"
Secalis + *Avenae* + *Tritici* varieties	1	"
Agrostidis + *Tritici* varieties	1	"
Agrostidis + *Poae* varieties	1	"
Poae variety alone	1	"

The predominance of the *Secalis* variety in these collections is probably the result of the ubiquitous presence of *Agropyron repens*, a grass susceptible to rye stem rust. Undoubtedly, aecia of wheat stem rust occur also each year on the barberry and are the means of disseminating that rust to such susceptible grasses or cereals as may be growing in the vicinity. The fact that, as the present studies have shown, a proportionally greater number of races occur in Eastern Canada than in the Prairie Provinces is perhaps a reflection of the role played by barberry in the production and dissemination of physiologic races. In Eastern Canada, 40 races occurred in 1013 isolates or one race for each 25.3 isolates. In the Prairie Provinces, 49 races were identified in 3475 isolates or one race per 70.9 isolates. Of the 40 races collected in Eastern Canada, 15 were not found in the Prairie Provinces. On the other hand, the collections made in the Prairie Provinces contained 24 races that have not been found in Eastern Canada. The collections made in British Columbia are too few to warrant comparison. However, in a total of 55 isolates there occurred three races not found in the adjacent Prairie Provinces.

From the figures given above, it is evident that there are many races that are not common to the Prairie Provinces and the rest of Canada. All of these, however, are races that occur only rarely. The races that are common in one region have always been found more or less commonly in the other regions. How these rare races originate and why they do not become permanent entities of the racial population are unsolved problems. In Eastern Canada and British Columbia, the races that have never been found in the prairie region may possibly be attributable to barberry. Such races could originate through selfing or crossing of wheat stem rust races, or, owing to numerous infections of barberry by rye stem rust, they could arise from crosses between wheat and rye stem rust. Races originating in such intervarietal crosses would probably, in most cases, be pathogenically weak on wheat (7).

Although most of the physiologic races now established in nature probably arose from barberry at some time or another, there can be little doubt that most new races produced on barberry do not become permanently established on cereals as economically important physiologic races. It may be inferred from the characteristics of many races produced in crossing and selfing studies

that some races originating on barberry are ill adapted for survival. Races produced on barberry differ from each other not only in pathogenicity but also in colour, abundance of urediospore production, and in the length of time elapsing between the commencement of uredial production and the formation of telia. It is almost certain that races with a long period of urediospore production have an advantage over races with a shorter period. Some races originating from barberry are known to have short uredial periods, a fact that would limit their abundance and distribution and would therefore militate against their permanent establishment in nature. The inheritance of this characteristic has not been studied extensively and is therefore not fully understood. Nevertheless, unpublished work by the writers has shown that some races with a brief uredial stage will give rise, when selfed, to races that vary considerably in the lengths of their periods of urediospore production, and may, consequently, after infection of barberry, produce races with uredial periods long enough to give them a good chance of survival. There exists, therefore, always the possibility that races not well adapted for survival may, through the instrumentality of the barberry, produce races that may survive and become important.

A characteristic of many races isolated from barberry, but which is not common in races collected on wheat or grasses, is the ability to attack certain emmer wheats. One such race that has attracted attention in recent years, though it has not yet become common, is race 15, and particularly its biotype 15*B*, which is pathogenic to some of the more recently developed rust resistant wheats (4). The possibility of the barberry playing a role in the origin of such highly pathogenic races should not be overlooked. There is, in fact, some evidence that races with ability to attack emmer wheats may originate in the selfing of races that lack this ability. Selfing studies by the writers with 14 races to which Vernal emmer wheat was highly resistant showed that seven of these races were heterozygous for ability to attack Vernal heavily; that is, they produced in their progeny some races to which Vernal was susceptible. Infection studies with teliospores collected on grasses have revealed similar races. For example, aecia on barberry inoculated with teliospores collected on *Agropyron trachycaulum* (Link) Malte var. *unilaterale* (Cassidy) Malte (*A. subsecundum* (Link) Hitchc.) gave rise to nine races of which four attacked Vernal heavily; and aecia derived from teliospores on *Agropyron trachycaulum* (Link) Malte var. *typicum* Fernald (*A. pauciflorum* (Schwein.) Hitchc.) produced 10 races of which five attacked Vernal moderately or heavily.

For some unknown reason, races that attack Vernal heavily have not as yet become established as important elements in the physiologic race population. In Canada, they are represented by only 108 out of 4543 isolates, or 2.4 % of all isolates. The fact that they are rather common in rust derived from barberry indicates the possibility of their spread and permanent establishment on cereals.

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OCCURRENCE OF *BACILLUS POLYMYXA* (PRAZ.) MIG. IN ALBERTA SOILS WITH SPECIAL REFERENCE TO ITS PATHOGENICITY ON POTATO TUBERS[1]

By A. W. Jackson and A. W. Henry[2]

Abstract

The spore-forming bacterium, *Bacillus polymyxa*, was found to occur widely in Alberta soils. All isolates obtained from the soil, together with several from other sources, proved capable of rotting potato tubers when introduced through wounds under conditions of abundant moisture and high temperature.

In these studies pure cultures of *Bacillus polymyxa* rotted potato slices at temperatures from 20° C. to 45° C. and whole tubers at temperatures from 30° C. to 45° C. but caused no damage below the minimum temperatures mentioned.

Though potato tubers and other vegetables that are formed in the soil probably often come in contact with *Bacillus polymyxa*, they are not likely to be rotted by it except when temperature and other factors are favourable. In general, soil temperatures in the field in Alberta are not conducive to the decay of potatoes by this organism, but those near the soil surface may on occasion reach favourable levels. The fact that *B. polymyxa* can be isolated quite frequently from rotted stored potatoes indicates that it is of some importance as a cause of decay in harvested potatoes. If, however, recommended storage temperatures are maintained in the storage quarters no damage from this organism should occur there.

Introduction

Bacillus polymyxa is a bacterium that is better known for its ability to ferment starch to form chiefly 2,3-butanediol and ethyl alcohol than as a plant pathogen. This organism has received some attention during the war just ended as a means of producing the above compounds, which have been useful in the war effort (7).

The idea that plant pathogenic bacteria are not spore-formers appears to have been quite generally accepted. However, a number of workers have reported certain spore-bearing bacteria to be capable of rotting vegetables and fruits. According to Brierley (4), Lepoutre in 1902 reported *Bacillus mesentericus vulgatus* to be capable of attacking potato tubers and Van Hall found *B. subtilis* and *B. vulgatis* pathogenic to potato tubers and other vegetables when incubated at 37° C. and 42° C. Brierley (4) isolated cultures of *B. mesentericus* and found them to be capable of rotting potato tubers over a wide range of temperature. Cultures used to inoculate the tubers were previously pasteurized at 80° C. for 15 min., thus assuring that they originated from spores. In 1943, Dowson (6) reported the isolation of *B. polymyxa* from a sample of rotting potato. This isolate proved capable of rotting slices of fresh potato, carrots, onions, cucumbers, and iris stems. When the organism was re-isolated from the rotted vegetables and inoculated into fresh slices, these were completely rotted. Decay took place from room

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temperatures up to 37° C. About the same time Madhok and Ud-Din (8) found a hitherto undescribed spore-forming bacterium capable of causing a fast-spreading rot in tomato fruits. This organism, which is Gram-positive and strictly aerobic, was named *Bacillus frutodestruens*. More recently, Allen (1) has reported a strain of *Bacillus subtilis* to be capable of rotting potato cubes submerged in nutrient broth. In this latter case the conditions employed departed rather widely from those that potatoes are likely to encounter in the hands of the grower.

The main objectives of this study were to determine whether Alberta soils contain plant pathogenic strains of *B. polymyxa*, to ascertain the prevalence of such strains, and to determine under what conditions they may be destructive to potato tubers.

Materials and Methods

Methods of Isolation

The methods used in the isolation of *B. polymyxa* were, in general, similar to those employed by Ledingham *et al.* (7) and were based upon the ability of this organism to form spores and to ferment carbohydrates rapidly with the evolution of gas. In addition, the production of detectable amounts of acetylmethylcarbinol and considerable quantities of 2,3-butanediol was considered indicative of the presence of *B. polymyxa* when selecting this species from other bacteria capable of fermenting starchy material with the production of gas.

Soil, or other material to be tested for the presence of *B. polymyxa*, was treated as follows.

Samples of the material were suspended in sterile water and shaken for two or three minutes. Aliquots (1 ml.) of the sediment-free liquor were added, one per tube, to test-tubes of 14 mm. bore each containing 5 ml. of sterile water. These prepared tubes were then pasteurized by immersion in a boiling water-bath for 10 min. Samples (1 ml.) of the pasteurized material were used to inoculate 10 to 12 ml. lots of sterile wheat mash.

The wheat mash was prepared by placing 0.8 gm. of ground wheat in a test-tube and adding 10 to 12 ml. of water. The contents of the tube were then thoroughly mixed by shaking and were allowed to stand at room temperature for one and one-half to two hours. The tubes so prepared were then plugged and autoclaved for 30 min. at 15 lb. pressure.

After incubation at 35° C. for two to four days, mashes containing *B. polymyxa* show vigorous fermentation with the evolution of much gas, and when fermentation has proceeded for four to five days, negative iodine tests for starch are usually obtained.

When spent mashes showed positive tests for acetylmethylcarbinol using the method of O'Meara (9) and yields of 1.5 to 2% 2,3-butanediol, using the method developed by Corns (5), it was concluded that they contained *B. polymyxa*.

Dilution plates were prepared from the contents of the fermentation tubes using neutral red agar as employed by Ledingham *et al.* (7). Colonies of *B. polymyxa* absorb the dye from the agar and hence are easily distinguished from those of certain other bacteria that may be present. After following the routine just described the culture obtained at this stage is usually almost pure *B. polymyxa*.

The colonies selected from the above agar were streaked onto wheat-peptone-agar slants, which were found satisfactory for the propagation of *B. polymyxa*. The composition of this medium except for the wheat is as follows: agar—17 gm., peptone—3 gm., and water— 1000 ml. These materials were heated in an autoclave for 5 to 10 min. at 15 lb. pressure. Tubes of the medium were then prepared by adding 0.5 gm. finely ground wheat to each, followed by 7 to 8 ml. of the liquefied peptone and agar mixture. Sterilization for 20 min. at 15 lb. steam pressure, cooling, and slanting of the medium were the final steps.

Method of Determining Pathogenicity

Potato tubers were used in the pathogenicity tests. These were prepared and inoculated as follows.

Potato slices were prepared from whole, sound potato tubers that had been previously washed in water to remove all dirt and then surface-sterilized in a 1:1000 mercuric chloride solution. After removal from the solution the tubers were thoroughly rinsed in sterile water. Slices about one-quarter to one-half inch thick were removed from the tubers with a sterile knife and placed in sterile Petri plates containing a single layer of filter paper and 5 ml. water. Potato slices were inoculated by smearing the freshly cut surface with bacteria suspended in sterile water. The suspension was prepared by transferring a large loopful of the bacterial growth from a 24-hr.-old, wheat-peptone-agar slant culture of the organism to 5 ml. of sterile distilled water. When whole potatoes were employed, they were placed, following surface sterilization, in desiccators containing a few millilitres of water to prevent drying. The desiccators were then placed in thermo-regulated chambers at the desired temperatures for 24 hr. previous to inoculation to bring the potatoes to the cabinet temperature. Tubers were inoculated by stabbing with a flamed inoculating needle carrying bacteria obtained from a 24-hr.-old slant of the desired organism.

After inoculation, the tubers and slices were incubated for three to four days, after which notes were taken on their condition.

Identification

The strains of *B. polymyxa* used in these studies fit the description given for this species by Bergey *et al.* (3) and by Porter, McCleskey, and Levine (10). The classification of Bergey *et al.* (3), which lists this organism as *Bacillus polymyxa* (Prazmowski) Migula, is followed here.

The identification is based on starch hydrolysis, formation of terminally located spores, negative Gram stain reaction, production of large quantities of 2,3-butanediol, and detectable amounts of acetylmethylcarbinol from carbohydrates. In addition the organism reduces nitrates to nitrites and produces acid, carbon dioxide, and hydrogen from a number of carbohydrates.

Plant Pathogenic Strains of *B. polymyxa* in Alberta Soils

Occurrence and Prevalence

Using the methods of isolation outlined above, the presence of *B. polymyxa* in Alberta soils was readily demonstrated. As indicated in Table I, it was obtained from all four of the main soil types of the province and from widely scattered points. Moreover, isolates made from a wide range of soil sources proved to be pathogenic to potatoes as was demonstrated by artificial inoculations, the results of which are recorded in the following and in subsequent sections.

TABLE I

Strains of *Bacillus polymyxa* found capable of rotting potato slices at 30° C. and sources of the isolates

American Type Culture Collection isolates	N.R.C.* isolates	University of Alberta isolates			
		From leached grey wooded soils	From black soil	From dark brown prairie soil	From brown prairie soil
840 7047 7070	C25 C42(2) C47 C56(2)	U.A.175 U.A.178	U.A.228 U.A.634 U.A.641 U.A.647 U.A.650 U.A.646(2)	U.A.152 U.A.155 U.A.167 U.A.183 U.A.186 U.A.197 U.A.234 U.A.236	U.A.179 U.A.201 U.A.219 U.A.220 U.A.222

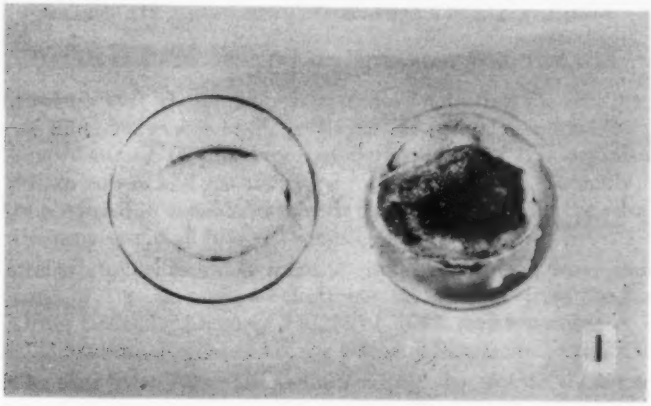
* *National Research Council of Canada.*

Note: *Sources of University of Alberta (U.A.) isolates: U.A. 152, Calgary; U.A. 155, Calgary; U.A. 167, Drumheller; U.A. 175, Dapp; U.A. 178, Perryvale; U.A. 183, Lethbridge; U.A. 186, Lethbridge; U.A. 197, Strathmore; U.A. 179, Taber; U.A. 201, Duchess; U.A. 219, Tilley; U.A. 220, Tilley; U.A. 222, Bow Island; U.A. 228, Olds; U.A. 234, Raymond; U.A. 236, Pincher Creek; U.A. 634, Edmonton; U.A. 641, Edmonton; U.A. 647, Edmonton; U.A. 650, decayed wood, Edmonton; U.A. 646(2), storm sewer discharge, Edmonton.*

Explanation of Figures

Fig. 1. *Characteristic appearance of potato slices rotted by Erwinia carotovora (left) and Bacillus polymyxa (right).*

Fig. 2. *The effect of temperature upon the ability of three strains (left U.A.641; centre, C42(2); right, American Type Culture 7070) to rot whole potato tubers. Bottom row, 25°; second row, 30°; third row, 35°; top row, 45° C.*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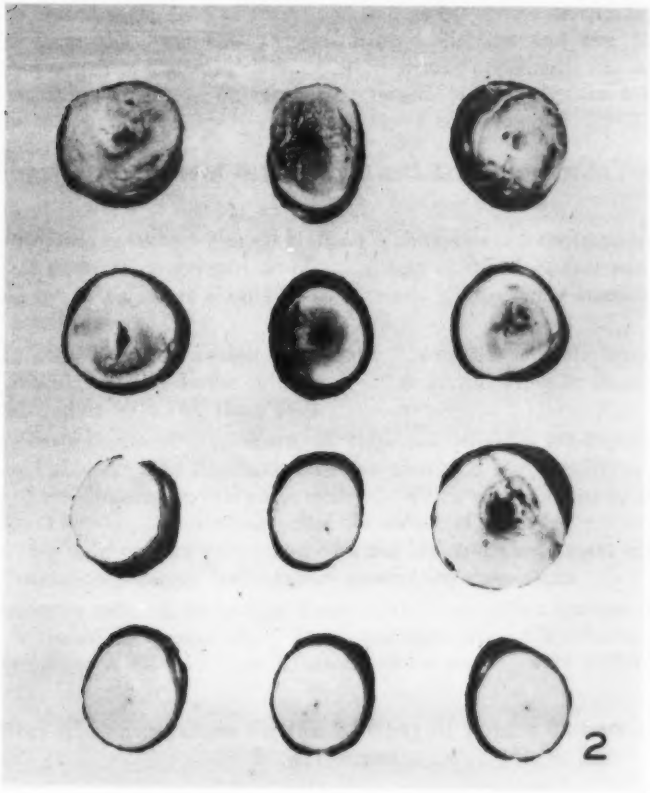
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Relative Pathogenicity of Different Soil Isolates

Potato tubers were chosen as host material for testing the relative pathogenicity of different strains of *B. polymyxa* for several reasons. Firstly, as noted above, Dowson (6) has reported the isolation of this organism from rotting potato tubers and its ability to rot potato slices. Secondly, potato tubers are produced in close contact with the soil, and thirdly they are grown all over the province in all four soil zones.

The strains studied included some 20 isolates obtained from soils from various parts of Alberta, and for comparison three strains from the American Type Culture Collection, namely 840, 7070, 7047, and a number of isolates made available from the collection of the National Research Council at Ottawa.

It can be seen from Table I that the ability to rot potato slices under the conditions of these experiments is possessed by all Alberta isolates of *B. polymyxa* tested, regardless of source, as well as by strains of this organism obtained from the American Type Culture Collection and the National Research Council of Canada. The different strains appeared to be approximately equal in virulence. Pathogenicity towards potato tuber tissue appears therefore to be quite generally characteristic of this organism.

Comparative Effects of *B. polymyxa* and *E. carotovora* on Potato Tubers

Since *Erwinia carotovora* (Jones) Holland is the cause of a common bacterial soft rot of potatoes, a comparison of the effects of this organism with those produced by *B. polymyxa* should be of interest. These were studied under similar conditions.

Potato slices prepared as described under "Materials and Methods" were surface inoculated with active cultures of either *E. carotovora* or *B. polymyxa* and incubated at 30° C. for three days.

In the early stages of breakdown, *B. polymyxa* caused a jell-like rot filled with gas bubbles. The production of gas caused a slight increase in the volume of the slice during this stage of decay, which was followed by collapse (Fig. 1). The slices, depending upon the strain of *B. polymyxa* used, are reduced to a light or dark grey-coloured mass, which darkens upon ageing to almost black. A pleasant fruity odour arises from these slices.

E. carotovora reduced the potato tissue to the well-known creamy mass of a more or less sticky consistency. The breakdown product is cream-coloured and, when no other bacteria than *E. carotovora* are present, very little odour is produced.

Effect of Temperature on the Rotting of Potato Tubers by *B. polymyxa*

Since temperature is one of the main factors to be considered in the storage of potatoes, it was decided to determine the temperature range over which potatoes are attacked by strains of *B. polymyxa*. The strains used included

representatives of those obtained from the American Type Culture Collection and the National Research Council of Canada, and two Alberta isolates.

Both whole and sliced raw potatoes were included in the temperature studies. These were prepared as previously described and were subjected to the temperatures indicated in Table II.

TABLE II

RELATIVE DEGREES OF ROTTING OF POTATOES BY *Bacillus polymyxa* AT DIFFERENT TEMPERATURES

Bacillus polymyxa strains used	Incubation temperature, °C.							
	15	20	25	30	35	40	45	50
Potato slices								
Check	−	−	−	−	−	−	−	−
7070	−	Tr.	+	+ +	+ + + +	+ + + +	+ + + +	−
C42(2)	−	−	+	+ +	+ + +	+ + +	+ + + +	−
U.A.641	−	Tr.	+	+ +	+ + + +	+ + + +	+ + + +	−
U.A.646(2)	−	−	+	+ +	+ + + +	+ + + +	+ + + +	−
Potato tubers								
Check		−	−	−	−	−	−	−
7070		−	−	+	+ + + +	+ + + +	+ +	−
C42(2)		−	−	−	+ +	+ +	+ +	−
U.A.641		−	−	−	+ +	+ +	+ +	−
U.A.646(2)		−	−	−	+ +	+ +	+ +	Tr.

NOTE: *Severity of rotting indicated as follows: − none; + slight to ++++ complete; tr. occasionally slight.*

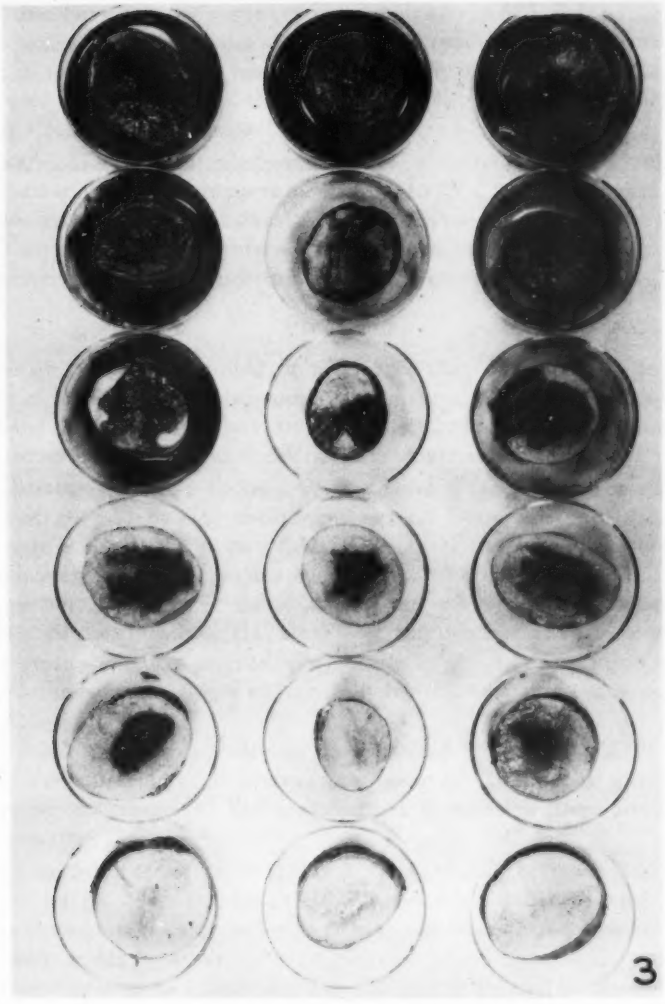
It is apparent from Table II that pure cultures of *B. polymyxa* are capable of rotting potato tuber tissue only at medium to high temperatures. Potato slices were rotted at temperatures from 20° to 45° C., whereas whole tubers were not rotted at temperatures below 30° C. The reason for this difference is not known (See Figs. 2 and 3).

Unpublished results obtained in this laboratory indicate that certain strains of *B. polymyxa* are capable of fermenting wheat mashes at temperatures as low as 15° C. This indicates that *B. polymyxa* may be chemically active at temperatures considerably below 20° C. However, from the results shown in Table II, it appears that *B. polymyxa* is unable by itself to attack potato tissue at such temperatures. In fact, temperatures exceeding 30° C. would appear necessary before much damage to whole tubers could be produced by this organism. The maximum temperature at which the tuber slices and whole tubers were consistently rotted was 45° C.

EXPLANATION OF FIGURE

FIG. 3. *Effect of temperature upon the ability of three strains (left, American Type Culture 7070; centre, C42(2); right, U.A. 641) to rot potato slices. Bottom row, 15°; second row, 20°; third row, 5°; fourth row, 30°; fifth row 35°; top row, 45° C.*

PLATE II



Isolation of *B. polymyxa* from Naturally Infected Potato Tubers

B. polymyxa has been isolated a number of times from rotting potato tubers that have been submitted to this laboratory. Although no instances of infection with this organism alone have been encountered in the material, it has, in most cases, been found in the actively rotting areas. The technique used for isolation was that described earlier in this paper under the heading "Methods of Isolation".

In addition, freshly cut portions of tubers, when buried in well moistened Edmonton soil in pots at temperatures of 20° to 25° C., became infected with *B. polymyxa* after four to six days' exposure. In one or two instances typical rot as caused by pure cultures of the organism was observed, but usually this bacterium occurred in mixtures with other bacteria and fungi.

Discussion

Although it is demonstrated in this paper that strains of *B. polymyxa* capable of rotting potato tubers occur commonly in Alberta soils, this should not be the cause of undue alarm since it is also shown that this organism can rot potatoes only under rather restricted environmental conditions.

Temperature is shown to be a very important factor in influencing the ability of *B. polymyxa* to rot potato tissue. Fortunately this organism apparently is able to rot potato tubers only at relatively high temperatures. Potatoes may become contaminated with it in the field, but are not likely to be severely rotted by it there, at least under average soil conditions in Alberta. Although records (11) show that, to a depth of a half inch, the soil in this region sometimes reaches temperatures at which this organism can rot potatoes, the moisture supply will generally be too low at such times to permit damage.

It is possible, of course, since we have isolated this organism from mixed infections of tubers stored at temperatures below 20° C., that this species may be of more importance at low temperatures in mixed cultures with other organisms than alone.

It has recently been reported (12) that, where heating occurs in field-stored potatoes, temperatures as high as 44° C. are reached and that much of the damage done in such instances is due to bacterial action. *B. polymyxa*, in all probability, would be active.

Another factor to be considered, and one that is affected by temperature, is wound cork formation. Artschwager (2) has shown that suberization takes place at the surface of freshly cut tubers within 24 hr. at 21° C. It has been observed in the course of these studies that potato slices stored for a few hours after cutting are then not readily rotted by *B. polymyxa*. It is quite possible that the formation of wound cork may be, in considerable measure at least, the explanation for this.

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INVESTIGATIONS ON RUBBER-BEARING PLANTS

II. CARBOHYDRATES IN THE ROOTS OF *TARAXACUM KOK-SAGHYZ* ROD.[1]

By Paul R. Gorham[2]

Abstract

The soluble carbohydrates were extracted, by means of hot water, from dried ground roots of *Taraxacum kok-saghyz* Rod. that had been extracted previously with acetone and benzene. A cleared portion of the extract served for the determination of hexose and, after invertase hydrolysis, sucrose. Another portion was subjected to mild acid hydrolysis before clearing, and served for the determination of total reducing value, whence fructosans, as inulin, were calculated. Separation of the free reducing sugars and sucrose from the fructosans by ethanol extraction proved unsatisfactory. Analyses of 171 one-year old roots from six crosses gave the following average values expressed as per cent dry weight: hexose 1.6, sucrose 4.7, and inulin 41. The analytical data suggest the possibility of selecting and breeding for strains of kok-saghyz capable of high carbohydrate production.

Introduction

The physiological and biochemical aspects of carbohydrate production and change in *Taraxacum kok-saghyz* Rod. have aroused considerable interest in connection with agricultural practices used with this plant. The carbohydrates have been of industrial concern as well, since their accumulation in the root is closely associated with that of rubber, and the carbohydrate content is sufficiently high to suggest alcohol production as a valuable adjunct to the process of rubber extraction. A recent review (13) covers much of the literature dealing with carbohydrates in kok-saghyz.

The carbohydrates of one-year old plants are mainly represented by inulin and reportedly comprise 12.6% on a fresh weight basis or 42.0% on a dry weight basis (4, 12). One ton of fresh roots yields about 60 litres of alcohol (4). It is estimated that for every ton of crude rubber extracted from kok-saghyz, two tons of alcohol (expressed as absolute alcohol) can be obtained from the inulin extracted by diffusion batteries of the type used for sugar beet (12).

The carbohydrate changes in kok-saghyz associated with development, maturation, and periodic fertilizer applications have been investigated (2, pp. 56–70; 8–11; 14, pp. 89–98; 18; 19; 20–22; 23, pp. 5–29). During the period of intensive growth, leaf carbohydrate content is low. With biological maturation, the rate of photosynthesis slows down and soluble carbohydrates accumulate in the leaves; inulin in the roots. This is accompanied by increased formation and storage of rubber. Supplying nitrogen at maturity stimulates further growth of the leaves, and consequently decreases the accumulation of

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both rubber and carbohydrates in the roots. However, rubber formation and accumulation bears no direct relation to photosynthesis (25–27).

After freshly harvested roots have been stored for two months, the inulin almost completely disappears and a considerable accumulation of mono- and disaccharides takes place (3). The exhaustion of carbohydrate reserves by developing leaves of kok-saghyz inhibits root initiation (24). The degree of survival of cuttings from roots in storage is correlated with their carbohydrate content (17).

Physiological studies concerned with the propagation of kok-saghyz by means of root cuttings made it desirable to have a procedure for carbohydrate analysis that would be suitable for routine application.

Materials and Procedure

Roots of individual seedlings from six crosses (four reciprocal), grown in an outdoor plot at Ottawa for five months prior to harvest*, were used for the study of carbohydrate content. These were dried at 40° C. and stored until analyses were undertaken. Each root was ground in a Wiley cutting mill, Intermediate Model, to pass a 20-mesh screen. The samples ranged in size from 1 to 5 gm. A few samples exceeding 5 gm. in weight were analysed in parts and the results totalled. All samples were extracted successively with acetone and benzene for resin and rubber hydrocarbons and the residual solvent allowed to evaporate before extraction of carbohydrates was undertaken.

Initially, attempts were made to separate the carbohydrates according to the method of Loomis and Shull (16, p. 281). This involved Soxhlet extraction for six hours with 85% ethanol for the removal of free reducing sugars and sucrose, followed by five successive hot water extractions for the removal of fructosans. This method was found to have several disadvantages, but these were overcome by adopting a procedure employing only hot water extraction.

Each sample, wrapped in a fluted filter paper as removed from the Soxhlet thimble, was placed in a 250 ml. beaker and 50 ml. of boiling water poured over it. This was placed on a multi-heat hotplate and maintained, with constant stirring, at a temperature of 90° to 95° C. for 30 min. The hot extract was carefully decanted, with as little transfer of sample as possible, into a funnel fitted with a 15 cm. fast-filtering paper. The filtrate was collected in a 250 ml. volumetric flask. Approximately 40 ml. of boiling water was added to the residue in the beaker and heated for another 15 min. This extract was then decanted into the same filter. The process was repeated three times more, after which experimental determination showed that little, if any, carbohydrate remained. With the last extraction, the sample was transferred to the filter and washed with a small amount of boiling water.

** Crosses were made and seedlings started in the greenhouse during the winter months of 1942–43. This work, as well as the subsequent analyses of the roots for resin and rubber hydrocarbons, was conducted by Dr. W. H. Minshall.*

The combined filtrates (I) were cooled, adjusted to volume, and aliquots withdrawn for analyses. Root samples of less than 1 gm. in weight were extracted in a total of 100 ml. instead of 250 ml. Routine extractions and analyses were conveniently carried out on lots of six samples.

Fifty millilitres of I were cleared and decolorized according to the method of Hassid (6). The amounts of saturated neutral lead acetate and disodium phosphate recommended by Hassid for clearing ethanol-soluble extracts were found to cause low reducing values when applied to aqueous extracts. Possibly an excess of phosphate interfered with the stoichiometric reduction of ferricyanide (1). Satisfactory reducing values, as determined by recovery experiments, were obtained by the use of 1 ml. of saturated neutral lead acetate followed by 2 ml. of saturated disodium phosphate. The cleared and decolorized extract (II) was made up to 100 ml., and served for determinations of sucrose and free reducing sugars.

Twenty-five millilitres of I were pipetted into a 50 ml. Erlenmeyer flask, 0.25 ml. of concentrated hydrochloric acid added, and the flask suspended for 35 min. in a water-bath at 70 $\pm$ 0.2° C. The acidified extract was cooled to room temperature, neutralized to litmus with 3*N* sodium hydroxide*, cleared and decolorized as above, and made up to 100 ml. This acid-hydrolysed extract (III) served for the determination of inulin.

The hydrolysis of the fructosans was carried out prior to clearing in order to avoid the possibility of inulin being carried down with the lead precipitate. Conditions of mild acid and moderate temperature were employed in order to minimize the hydrolysis of pectin, gums, or glucosans that were present. These conditions were found adequate, however, for the complete hydrolysis of sucrose, thereby providing a means for the determination of the total reducing value.

The ferricyanide–ceric-sulphate method of Hassid (7) was employed for the determination of reducing sugars. Replicate analyses carried out initially agreed so closely that for routine purposes single determinations for each carbohydrate were deemed adequate. Aliquot sizes were reduced and runs repeated whenever heating produced complete decolorization of the ferricyanide.

Hexose

The amount of free reducing sugars, expressed as hexose, was determined on a 5 ml. aliquot of II.

Sucrose

Hydrolysis of 25 ml. of II was carried out in a 50 ml. volumetric flask. The pH was adjusted to 4.5 with 10% acetic acid, using methyl red as internal indicator, and two drops of a 1% aqueous suspension of invertase were then

* *About 0.75 ml. were required. In most cases, the end-point of the titration was determined by the appearance of the pronounced yellow colour of a naturally occurring indicator. Litmus was used only when some obscuring pigment made this end-point uncertain. Excess alkali was found to interfere with clearing and to cause greatly reduced analytical values.*

added. After 22 hr. at room temperature, sufficient for complete hydrolysis, the volume was made up to 50 ml. and the combined reducing value of the hydrolysed sucrose plus free reducing sugars was determined on an aliquot of 5 ml. A correction was applied for the slight reducing value of the invertase. The sucrose content was determined by multiplying the difference between the free reducing value and the combined reducing value per sample by 0.95, the ratio of one gram-mole of sucrose to two gram-moles of hexose.

Inulin

The total reducing value was determined on a 1 ml. aliquot of III. The fructosan content, expressed as inulin (assuming the formula $(C_6H_{10}O_5)_6 . H_2O$), was determined by multiplying the difference between the combined reducing value and the total reducing value per sample by 0.916.

Results and Discussion

In the initial trial of the method of Loomis and Shull for separating the carbohydrates the first disadvantage was that the aqueous extract following ethanol extraction was found to contain hexose to the extent of 9 to 19% of the total found in both extracts (Table I). This would have necessitated a hexose determination on each extract.

TABLE I

TOTAL HEXOSE CONTENT OF DRIED KOK-SAGHYZ ROOTS DETERMINED BY ETHANOL EXTRACTION FOLLOWED BY WATER EXTRACTION

Sample	Dry wt., gm.	By ethanol, %	By water, %	Total, %	Per cent of total in water extract
S64.1	4.406	1.20	0.29	1.49	19
.2	3.235	1.35	0.13	1.48	9
.3(*a*)	4.356	1.14	0.21	1.36	15
(*b*)	3.452	1.29	0.28	1.57	18
.4	1.809	2.48	0.31	2.79	11

The second disadvantage was that part of the fructosans were extracted by the 85% ethanol and erroneously determined as sucrose. Evidence of this was provided by the observation that sucrose values obtained after invertase hydrolysis of ethanol-soluble extracts were appreciably lower than those obtained after acid hydrolysis. The observed differences ranged from 1.5 to 4.3% (Table II), and were not caused by incomplete hydrolysis with invertase since values determined after 40 hr. differed little from those determined after 24 hr.

A third disadvantage, from the standpoint of routine analysis, was the additional time required for the two types of extraction.

The maximum, minimum, and mean values, in per cent, for the hexose, sucrose, inulin, and total soluble carbohydrates found in the seedling roots

from six crosses are summarized in Table III. The hexose content was consistently low in all crosses, with mean values ranging from 1.3 to 2.2%. Attempts to evaluate the glucose and fructose content by means of a modified

TABLE II

SUCROSE DETERMINATIONS UPON THE ETHANOL-SOLUBLE EXTRACT OF DRIED KOK-SAGHYZ ROOTS AFTER HYDROLYSIS WITH HYDROCHLORIC ACID OR WITH INVERTASE

Sample	Dry wt., gm.	Sucrose, %			Difference, %
		Hydrochloric acid	Invertase		
			24 hr.	40 hr.	
S66.19	3.564	13.0	9.7	9.6	3.3
.20	1.851	13.1	8.8	8.6	4.3
.21	2.892	11.9	9.2	9.1	2.7
.22	2.093	8.9	7.4	7.0	1.5
.23	3.074	6.8	4.7	4.8	2.1
.24	0.849	13.1	8.8	8.5	4.3

TABLE III

MEAN VALUES, TOGETHER WITH THE MINIMUM AND MAXIMUM VALUES OBSERVED, FOR THE HEXOSE, SUCROSE, INULIN, AND TOTAL SOLUBLE CARBOHYDRATES CONTENT OF SEEDLING ROOTS FROM SIX CROSSES OF *Taraxacum kok-saghyz*

Cross	No. roots analysed	Dry wt., gm.	Hexose, %	Sucrose, %	Inulin, %	Total sol. carbohydrates, %
253.2 × 181.8	24	2.243 0.659–6.263	1.9 0.8–3.0	4.7 2.1–8.7	41.3 21.6–51.3	47.9 28.1–57.3
253.2 × 253.3	6	2.068 1.279–3.156	1.5 1.2–1.8	6.3 5.7–6.8	46.9 46.0–48.1	54.7 53.4–56.3
253.2 × 257.1	32	1.121 0.083–4.398	1.4 0.6–2.4	5.3 1.8–10.5	43.0 23.8–50.7	49.7 27.1–57.2
	21	3.087 0.659–6.195	1.9 1.3–3.0	5.4 3.2–8.9	40.9 38.1–48.1	48.2 44.9–57.0
257.1 × 93.24	14	1.074 0.073–3.072	1.3 0.5–2.4	3.9 1.4–6.1	41.8 33.4–53.2	47.0 39.2–59.6
	17	1.746 0.075–3.057	1.5 0.5–2.4	3.3 0.3–4.7	41.7 29.1–59.5	46.5 35.5–62.2
93.24 × 150.11	5	1.118 0.318–1.894	1.5 1.3–1.8	2.0 0.0–3.9	33.9 24.2–37.8	37.4 27.0–42.5
	13	2.110 0.843–3.025	1.7 1.0–2.1	6.5 4.3–8.3	32.7 23.1–40.4	40.9 33.1–48.5
150.11 × 253.2	5	1.397 0.408–3.586	2.2 1.4–3.6	–	40.2* 30.4–47.7*	42.5 32.0–49.1
	34	2.388 0.198–6.154	1.5 0.8–2.7	–	42.7* 13.3–63.9*	44.2 15.0–64.6

* *Combined sucrose and inulin.*

ferricyanide procedure resembling that of Becker and Englis (1, 5) were not wholly satisfactory. However, the results indicated the presence of both glucose and fructose in either approximately equal proportions or with fructose predominating. Throughout the various crosses the mean contents ranged from 2.0 to 6.5% for sucrose, 32.7 to 46.9% for inulin, and 37.4 to 54.7% for the total soluble carbohydrates.

The mean values found for the three types of carbohydrate compare favourably with those reported by others (8, 18, 22). The sucrose and inulin content exceeds that found in the roots of our native dandelion, *Taraxacum officinale* Weber (15). This species contains reducing sugar 1.2%, sucrose 3.5%, and polysaccharides, consisting of approximately equal parts of fructosan and dextrin, 10.7%, calculated on a dry-weight basis. There is little or no inulin present. In kok-saghyz, the fraction reported as inulin undoubtedly contained a considerable proportion of lower molecular weight fructosans. This is suggested by the ethanol-soluble, acid-hydrolysable carbohydrate that was unaffected by invertase (Table II).

Differences in the growth of the seedlings, as evidenced by variation in root dry weight, were considerable (Table III). However, carbohydrate content was not correlated with root weight. Although the mean values for the total soluble carbohydrates in reciprocal crosses agreed quite well, comparisons as to carbohydrate production by specific crosses are of little significance because of the great variation in content and numbers of plants surviving within each cross. However, the data suggest that selecting and breeding for strains capable of producing large amounts of carbohydrate is feasible.

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